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Susceptibility/resistance status of malaria vector anophelines species in Bajaur agency, FATA

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Abstract
Plasmodium is the causative agent of malaria, transmit with the bites of female mosquito anopheles called malarial vectors. The four species *P. Falciparum, P. vivax, P. ovale* and *P. Malariae* are the causative agents of the malaria. Malaria is major cause of morbidity and mortality with high risk areas of Pakistan, mainly located in Sindh, Baluchistan, FATA and KPK province of the country. The present study was aim to determine the resistance status of malarial species especially Anopheles in Bajaur (FATA).The parasite causing infection in more than one third of global population or over 2 billion peoples living in more than 100 countries. It has been estimated that about 1.6 million cases may occur in Pakistan. The world health organization estimated that 59% of the world clinical malaria cases occur in Africa, 38% in Asia3% in the America nearly 5 million confirmed cases of malaria are reported each year from different countries outside of Africa. Out of which nearly 3 million have repotted from India and Pakistan. In total of 125% samples 20% was in control and 80% were divided into equal strata with regarding of the medicines, each group become 25% in the total of 100.

Keywords: Anopheles, *P. falciparum, P. vivax, P. ovale* and *P. Malariae,* mortality, morbidity

Introduction
Malaria is caused by plasmodium parasite, which spared through the bites of female mosquitoes [1]. There are more than 120 species of malaria parasite of *genus plasmodium* throughout the world; there are 4 species *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale* and *Plasmodium Malariae,* caused malaria [2]. Mosquitoes have 3500 species are grouped into 41 genera. Human malaria is caused by a female mosquito of genus anopheles [3]. Malaria is endemic in Pakistan with prevalence varying with season. In Pakistan major causes of malaria morbidity and mortality mostly present in area of Sindh, Baluchistan, and K.P.K and FATA province of the country. Some study reported that about 1.6 Million malaria cases occur in Pakistan [4]. *Plasmodium falciparum* is dangerous malaria parasite, responsible mostly for human mortality in the world. In 1998 WHO (World Health Organization) reported more new cases of malaria world, which are 300 to 500 million per year (reported in 1998) than there were in 1954 (estimated 250 millions). The estimated death rate of malaria in 1997 was between 1.5 million and 2.7 million, with time of period these cases are raised and or stable [5]. Malaria is an important vector born disease caused by Malaria parasite named *Plasmodium*. Malaria parasite causes infection in more than one third of global population, over 2 billion peoples living in more than 100 countries. The estimated clinical episodes of malaria cases is 300—500 millions of acute illness worldwide each year. The world health organization estimated that Africa 59% cases occur, 38% in Asia3% in the America nearly 5 million confirmed cases of malaria are reported each year from different countries outside of Africa. out of which nearly 3 million have repotted from India and Pakistan, countries where the malaria situations have remained more or less unchanged for the last decade [6] Malaria is estimated to cause deaths between 1.1 and 2.7million deaths annually worldwide, of which 90% occur in sub Saharan Africa mainly children under the age of 5 years are affecting which results for the 25% of child mortality in Africa [7].
Mostly malaria parasites are present in twenty countries. It is in eastern Asia, South east and in the western pacific.

In Asia more than 2 billons of people are risk for malaria but in worldwide population 67% of People at risk to malaria were reported. The ten largest countries of the Asia are more near to risk at malaria then other countries particularly areas of China, Indonesia, Bangladesh, Vietnam, and are some region of Philippines [8]. The malaria is controlled by the method of vector control approach [9].

For the malaria controlling some other strategy are uses like mostly insecticides to treated mosquitoes, Nets and others material indoor spray are used against mosquitoes. For the limitation of mosquitoes larviciding used pyrethroid insecticide to treat. Some studies show vector of malaria show resist to all four classes of insecticide due multiple using [10]. Mostly malaria is present in twenty countries in worldwide in region of Asia [11]. The most recently pyrethriod resistance were reported firstly and also across resistance to 'DDT' in An Gambia [12].

Dichlorodiphenyltrichloroethane (DDT)- Pyrethroid are used for control of malaria vector and show good result against vector in Africa, and DDT is one of the recommended insecticide for treat in bed net and for used in IRS [13].

During 1952 to 1974 DDT was the most effective insecticide the malaria vector. DDT spray killed the mosquitoes and causes to decrease man and vector contacts [14].

Latter then 1974 DDT shows resistance to vector and replaced by Dieldrin insecticides. After then in 1976 firstly in Punjab province Malathion insecticide were introduced. In 1980 Malathion insecticides was also failed *An. Stephensi* spp show resist in Lahore District [15].

During 1983 some study was revealed that spp Anopheline population has susceptible to mostly insecticides in District Faisalabad [16]. Due to insecticides Resistance no of malaria cases increasing day by day, due to which morbidity and mortality rates increases. So there is a need to find out suitable insecticides to decrease Anopheline population.

Materials and Methods

The following materials were used in this study

12 plastic tubes (125mm in length and 44 mm in diameter), with each tube fitted at one end with 16-mesh screen the 12 tubes include:

Four marked with a red dot for use as 'exposure tubes, i.e. for exposing mosquitoes insecticides impregnated papers.

Two marked with a yellow dot, as exposure tube, for exposure of mosquitoes to the oil treated control papers, i.e. Without insecticide

Six with a green dot for use as holding tubes, for pretest sorting and for post exposure observations.

Six slides units, each with a screw –cap on either side, provided with 20 mm filling hole.

40 sheets of clean papers (12×15 cm) for lining the holding tubes.

Insecticide-impregnated papers.

Instruction sheets.

A mouth aspirator.

The insecticide-impregnated papers are prepared only with the discriminating concentrations of relevant insecticides. These papers were packed in plastic boxes and each box contains 8 papers. In this research work Deltamethrin, Malathion, Bendiocarb and DDT insecticide-impregnated papers were used in the separated tubes.

Study Areas

The study areas were three Tehsils of Bajaur agency, named, Tehsil Nawagi, Mamond kasona and Tange Charmang bajaur agency,

Study duration

Study time period was Oct 2013 to December 2013.

Study population

Sample of mosquitoes was taken from three different Tehsils of bajaur agency with high density of vector population. Sample size was 125 fully feed mosquitoes. Four replicates and one control was run up against each insecticide. Each replicate was containing 25 mosquitoes. Control was also containing 25 mosquitoes.

Collection Technique of mosquitoes

Mosquitoes were collected from field in the morning 6 am to 8 am. Mosquitoes were collected through CDC sweeper and mouth aspirator.

Sample size

125 Mosquitoes were caught from field, 100 were kept in 4 exposure tubes and 25 in the control tube.

Mosquitoes were collected from three different Tehsils of Bajaur agency with the help of CDC sweeper and mouth aspirator. Collected mosquitoes were given immediately a moisture pad. These mosquitoes were brought from field to civil hospital laboratory of Nawagai bajaur agency. Sample size of mosquitoes was 125 of *An. Stephensi* after sorting out of 125, Hundred (100) feed *Anopheline* mosquitoes were exposed to insecticide –impregnated paper, in exposure tubes, marked with a red dot. There were four exposure tubes; each was containing 25 *Anopheline* mosquitoes.

A control was also run to point out the real cause of mortality of mosquitoes. The control tube was containing 25 fed *Anopheline* Mosquitoes. All the collected 125 mosquitoes were in the holding tubes and a plastic tube (lined with filter paper impregnated with mineral oil) was used as control, connected to holding tube. Mosquitoes were transferred from holding tube to the control tube through a hole in the slide between the two tubes. Similarly mosquitoes from other holding tubes were transferred to four plastic tubes lined with insecticide impregnated papers, called exposure tubes. Filter papers were hold in place by special metallic rings.

Slides were remained closed and exposure as well as control tubes, which were contain mosquitoes stand upright for one hour period. After one hour mortality rates were calculated and recorded. After this mosquitoes were back transferred to holding tubes, where they were kept for 24 hours, with a piece of moist cotton wool. Temperature and humidity were monitored.

After this recovery period mortality in exposure tubes as well as in the control tube was calculated and recorded.

At the end of test it was found that Deltamethrin was Susceptible and Malathion, Bendiocarb and DDT were resisting.

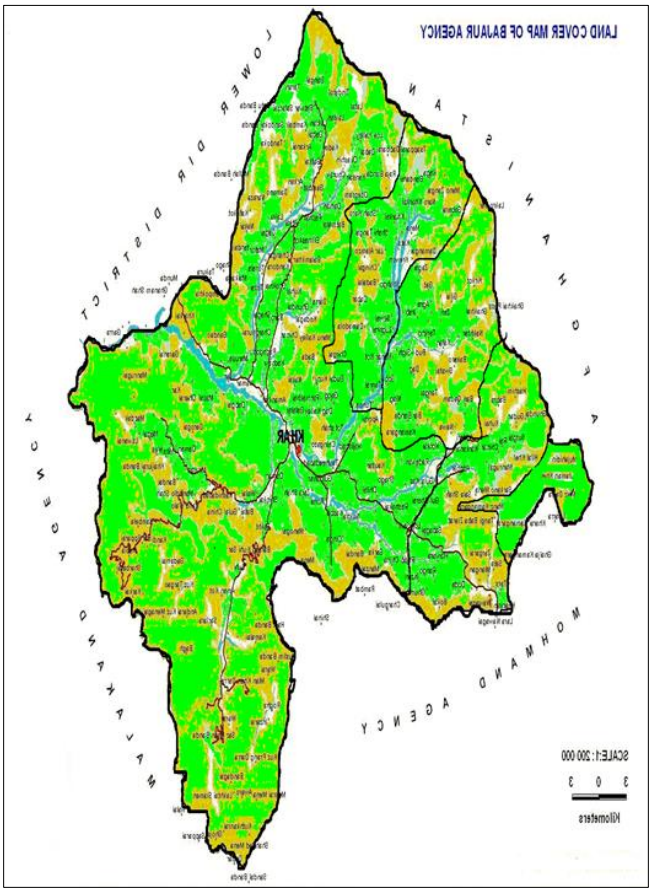

Fig 1: Bajaur Agency Map Border Length with Afghanistan = 65 Kilometers

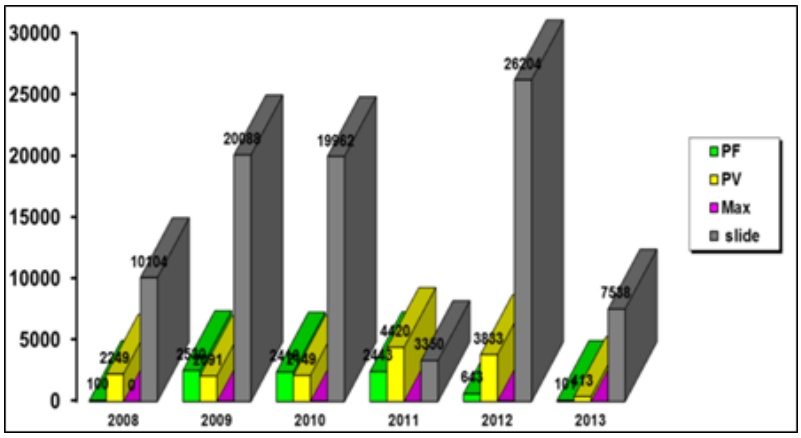

Fig 2: trend of positive cases of malaria in bajaur agency 2008-2013

Results

100 mosquitoes which were grouped into four separate tubes. Mortality and morbidity rate were checked which was shown in the following tables. The susceptibility/resistance levels in *Anopheles stephensi* to the different insecticides such as Deltamethrin, Malathion, Bendiocarb and DDT were tested. out of these Deltamethrin was susciptable while Malathion, Bendiocarb and DDT were resistance after 24 hours of incubation. Insecticide intensities and oxidative detoxification mechanism are shown in Tables 1-12 *Anopheles stephensi* was the only malaria vector tested in all the three Tehsils of Bajaur agency, named, Tehsil Nawagi, Mamond kasona and Tange Charmang bajaur agency, the three study site (Nawagi, Mamond kasona and Tange Charmang). *Anopheles stephensi* was tested in these districts. Results were collected from *An. stephensi* against 4% DDT. Test with diagnostic dose of DDT showed resistance in these three localities, percentage mortality ranged was from 55% to 59%. Malathion showed result 72% to 73% mortality which is in the range of Resistance. Bendiocarb mortality percentage was 41% to 45% which indicates Resistance. Deltamethrin was found susceptible which have showed 98% to 100% mortality. Tests were performed on *An. stephensi* against 0.5% of Deltamethrin which belong to pyrethroid group of insecticide. Test with diagnostic doses of Deltamethrin showed complete susceptibility throughout the three Tehsils of Bajaur agency.

Table 1: Susceptibility/Resistance statutes of *An. stephensi* against 0.05% Deltamethrin at Nawagai, Bajaur agency Concentration: 0.05%

Species tested	N0 tested		Mortality after 1 hr			Mortality after 24 hr		%age mortality of each replicate	Total mortality %age after 24 hr	Status
			Living	Dead		Living	Dead			
Anopheles Stephens	R1	25	8	17	65% 4%	0	25	100%	99%	s
	R2	25	11	14		1	24	96%		
	R3	25	7	18		0	25	100%		
	R4	25	9	16		0	25	100%		
	Total	100	35	65		1	99	-------		
	Control	25	24	1		24	1	4%		

S= Susceptible

Result of susceptibility/resistance test of *Anopheles stephensi* against 0.05% of deltamethrin at optimum temperature and humidity at Nawagai Bajaur agency is shown in table no 1. Results show that *An. stephensi* was susceptible against deltamethrin, showing mortality percentage of 99%.

Table 2: Susceptibility/Resistance statutes of *An. stephensi* against 0.05% Malathion at Nawagai, Bajaur agency: Concentration: 0.05%

Species tested	N0 tested		Mortality after 1 hr			Mortality after 24 hr		%age mortality of each replicate	Total mortality %age after 24 hr	Status
			Living	Dead		Living	Dead			
= *Anopheles stephensi*	R1	25	6	19	70% 0%	6	19	76%	73%	R
	R2	25	9	16		8	17	68%		
	R3	25	8	17		6	19	76%		
	R4	25	7	18		7	18	72%		
	Total	100	30	70		27	73	--------		
	Control	25	25	0		24	1	4%		

R=Resistance

Result of susceptibility/resistance test of *Anopheles stephensi* against 0.05% Malathion at optimum temperature and humidity at Nawagai Bajaur agency is shown in table no 1. Results show that *An. stephensi* was resistant against Malathion, showing mortality percentage of 73%.

Table 3: Susceptibility/Resistance statutes of *An. stephensi* against 0.05% Bendiocarb at Nawagai, Bajaur agency Concentration: 0.05%.

Species tested	N0 tested		Mortality after 1 hr			Mortality after 24 hr		%age mortality of each replicate	Total mortality %age after 24 hr	Status
			Living	Dead		Living	Dead			
Anopheles Stephens	R1	25	16	9	30% 4%	17	8	32%	43%	R
	R2	25	19	6		15	10	40%		
	R3	25	18	7		13	12	48%		
	R4	25	17	8		12	13	52%		
	Total	100	70	30		57	43	-------		
	Control	25	24	1		24	1	4%		

R=Resistance

Result of susceptibility/resistance test of *Anopheles stephensi* against 0.05% bendiocarb at optimum temperature and humidity at Nawagai Bajaur agency is shown in table no 4. Results show that *An. stephensi* was susceptible against bendiocarb, showing mortality percentage of 43%.

Table 4: Susceptibility/Resistance statutes of *An. stephensi* against 4% DDT at Nawagai, Bajaur agency.

Species tested	N0 tested		Mortality after 1 hr			Mortality after 24 hr		%age mortality of each replicate	Total mortality %age after 24 hr	Status
			Living	Dead		Living	Dead			
Anopheles stephensi	R1	25	16	9	37% 4%	13	12	48%	59%	R
	R2	25	15	10		10	15	60%		
	R3	25	14	11		8	17	68%		
	R4	25	18	7		9	16	64%		
	Total	100	63	37		41	59	-----		
	Control	25	24	1		24	1	4%		

R=Resistance

Result of susceptibility/resistance test of *Anopheles stephensi* against DDT at optimum temperature and humidity at Nawagai Bajaur agency is shown in table no 4. Results show that *An. stephensi* was susceptible against DDT, showing mortality percentage of 59%.

Table 5: Susceptibility/Resistance statutes of *An. stephensi* against 0.05% Deltamethrin at Tange charmang, Bajaur agency: Concentration: .05%

Species tested	N0 tested		Mortality after 1 hr			Mortality after 24 hr		%age mortality of each replicate	Total mortality %age after 24 hr	Status
			Living	Dead		Living	Dead			
Anopheles stephensi	R1	25	16	9	37% 4%	0	25	100%	100%	S
	R2	25	15	10		0	25	100%		
	R3	25	14	11		0	25	100%		
	R4	25	18	7		0	25	100%		
	Total	100	63	37		0	100	-------		
	Control	25	24	1		24	1	4%		

S=Susceptible

Result of susceptibility/resistance test of *Anopheles stephensi* against 0.05% Deltamethrin at optimum temperature and humidity at Nawagai Bajaur agency is shown in table no 5. Results show that *An. stephensi* was susceptible against Deltamethrin, showing mortality percentage of 100%.

Table 6: Susceptibility/Resistance statutes of *An. stephensi* against 0.05% Malathion at Tange, Bajaur agency. Concentration: 0.0

Species tested	No tested		Mortality after 1 hr			Mortality after 24 hr		%age mortality of each replicate	Total mortality %age after 24 hr	Status
			Living	Dead		Living	Dead			
Anopheles stephensi	R1	25	6	19	70% 0%	7	18	72%	69%	R
	R2	25	9	16		6	19	76%		
	R3	25	8	17		7	18	72%		
	R4	25	7	18		8	17	68%		
	Total	100	30	70		28	69	-----		
	Control	25	25	0		24	1	4%		

R=Resistance

Result of susceptibility/resistance test of *Anopheles stephensi* against 0.05% Malathion at optimum temperature and humidity at Nawagai Bajaur agency is shown in table no 6. Results show that *An. stephensi* was susceptible against Malathion, showing mortality percentage of 69%.

Table 7: Susceptibility/Resistance statutes of *An. stephensi* against 0.05% Bendiocarb at Tange charmang Bajaur agency: Concentration: 0.05%

Species tested	N0 tested		Mortality after 1 hr			Mortality after 24 hr		%age mortality of each replicate	Total mortality %age after 24 hr	Status
			Living	Dead		Living	Dead			
Anopheles stephensi	R1	25	19	6	29% 4%	16	9	36%	45%	R
	R2	25	17	8		14	11	44%		
	R3	25	18	7		13	12	48%		
	R4	25	17	8		12	13	52%		
	Total	100	71	29		55	45	-----		
	Control	25	24	1		24	1	4%		

R=Resistance

Result of susceptibility/resistance test of *Anopheles stephensi* against 0.05% bendiocarb at optimum temperature and Humidity at Tange charmang Bajaur agency is shown in table no 7. Results show that *An. stephensi* was susceptible against bendiocarb, showing mortality percentage of 45%.

Table 8: Susceptibility/Resistance statutes of *An. stephensi* against 4% DDT at Tange charmang, Bajaur agency: Concentration: 4%

Species tested	N0 tested		Mortality after 1 hr			Mortality after 24 hr		%age mortality of each replicate	Total mortality %age after 24 hr	Status
			Living	Dead		Living	Dead			
Anopheles stephensi	R1	25	11	14	62% 0%	10	15	60%	55%	R
	R2	25	10	15		11	14	56%		
	R3	25	8	17		12	13	52%		
	R4	25	9	16		12	13	52%		
	Total	100	38	62		45	55	55%		
	Control	25	25	0		24	1	4%		

R=Resistance

Result of susceptibility/resistance test of *Anopheles stephensi* against 4% DDT at optimum temperature and humidity at Tange charmang Bajaur agency is shown in table no 8. Results show that *An. stephensi* was Resistant against DDT, showing mortality percentage of 55%.

Table 9: Susceptibility/Resistance statutes of *An. stephensi* against 0.05% Deltamethrin at Mamond Kasona, Bajaur agency: Concentration: 0.05%

Species tested	No tested		Mortality after 1 hr			Mortality after 24 hr		%age mortality of each replicate	Total mortality %age after 24 hr	Status
			Living	Dead		Living	Dead			
Anopheles stephensi	R1	25	16	9	37% 4%	0	25	100%	98%	S
	R2	25	15	10		1	24	96%		
	R3	25	14	11		1	24	96%		
	R4	25	18	7		0	25	100%		
	Total	100	63	37		2	98	----		
	Control	25	24	1		24	1	4%		

S=Susceptible

Result of susceptibility/resistance test of *Anopheles stephensi* against 0.05% Deltamethrin at optimum temperature and humidity at mamond kasona Bajaur agency is shown in tale no 9. Results show that *An. stephensi* was susceptible against Deltamethrin, showing mortality percentage of 99%.

Table 10: Susceptibility/Resistance statutes of *An. stephensi* against 0.05% Malathion at mamond kasona Bajaur agency: Concentration: 0.05%

Species tested	No tested		Mortality after 1 hr			Mortality after 24 hr		%age mortality of each replicate	Total mortality %age after 24 hr	Status
			Living	Dead		Living	Dead			
Anopheles stephensi	R1	25	6	19	70% 4%	6	19	72%	72%	R
	R2	25	9	16		9	16	68%		
	R3	25	8	17		7	18	72%		
	R4	25	7	18		6	19	68%		
	Total	10	30	70		28	72	-----		
	Control	25	24	1		24	1	4%		

R=Resistance

Result of susceptibility/resistance test of *Anopheles stephensi* against 0.05% Malathion at optimum temperature and humidity at Mamond kasona Bajaur agency is shown in tale no 10. Results show that *An. stephensi* was susceptible against Malathion, showing mortality percentage of 72%.

Table 11: Susceptibility/Resistance statutes of *An. stephensi* against 0.05% Bendiocarb at Mamond kasona Bajaur agency: Concentration: 0.5%

Species tested	No tested		Mortality after 1 hr			Mortality after 24 hr		%age mortality of each replicate	Total mortality %age after 24 hr	Status
			Living	Dead		Living	Dead			
Anopheles stephensi	R1	25	18	7	72% 4%	17	8	36%	41%	R
	R2	25	15	10		14	11	48%		
	R3	25	17	8		15	10	48%		
	R4	25	18	7		13	12	52%		
	Total	100	68	72		59	41	-----		
	Control	25	24	1		24	1	4%		

R=Resistance

Result of susceptibility/resistance test of *Anopheles stephensi* against 0.5% bendiocarb at optimum temperature and humidity at Mamond kasona Bajaur agency is shown in table no 11. Results show that *An. stephensi* was susceptible against bendiocarb, showing mortality percentage of 41%.

Table 12: Susceptibility/Resistance statutes of *An. stephensi* against 4% DDT at Mamond kasona, Bajaur agency: Concentration: 4%

Species tested	N0 tested		Mortality after 1 hr			Mortality after 24 hr		%age mortality of each replicate	Total mortality %age after 24 hr	Status
			Living	Dead		Living	Dead			
Anopheles stephensi	R1	25	11	14	61% 0%	12	13	52%	55%	R
	R2	25	12	13		11	14	56%		
	R3	25	7	18		10	15	60%		
	R4	25	9	16		12	13	52%		
	Total	100	39	61		45	55	------		
	Control	25	25	0		24	1	4%		

R=Resistance

Result of susceptibility/resistance test of *Anopheles stephensi* against 4% DDT at optimum temperature and humidity at Mamond kasona Bajaur agency is shown in table no 12. Results show that *An. stephensi* was susceptible against DDT, showing mortality percentage of 55%.

Discussion

This study highlighted the distribution and frequency of insecticide resistance in the Anopheles known malaria vector in Bajaur (FATA), Pakistan, in order to better guide insecticide-based vector control. In Pakistan there are two main malaria vectors, *An. stephensi* and *An. Culicifacies*.

DDT resistance was found in *An. stephensi*, *An. culicifacies*, *An. subpictus* and *An. Annularus* but was susceptible in *An. pulcherimus* due to their exophalic nature in 1883 in district faisal Abad and many other district of Punjab[26]. In Mangalore city, the resistance was observed in *An. stephensi* to Malathion and low mortalities recorded. In deltamethrin and cyfluthrin due to selective pressure from fogging, IRS including anti-larval operation with these molecules for more than a decade [27].

A study conducted in Pakistan, that Anopheline population of this province was completely susceptible to the two organophosphates mention and feniltrothion, while Malathion was resistance, all Anopheline were completely susceptible to the Carbamate and propoxure [27].

Another study analyzed that population of *An. culicifacies* in Lahore has a very low frequency of susceptible gene. It was found that DDT use for mosquito control has been discontinued in that district and no reversal of DDT resistance has taken place [28]. Same study was conducted in which DDT was resistance to the malaria parasite [29]

In our study at Bajaur agency collection was made from three different localities of Bajaur agency. In Bajaur agency Deltamethrin is used as insecticide for killing malaria vector, which belong to pyrethroid. Tests were performed at three localities; Tehsil Nawagai, Tehsil mamond Kasona and Tehsil charming Tange of Bajaur agency. Results were collected from *An. stephensi* against 4% DDT. Test with diagnostic dose of DDT showed resistance in these three localities, percentage mortality ranged was from 55% to 59%. Malathion showed result 72% to 73% mortality which is in the range of Resistance. Bendiocarb mortality percentage was 41% to 45% which indicates Resistance. Deltamethrin was found susceptible which have showed 98% to 100% mortality. Tests were performed on *An. stephensi* against 0.5% of Deltamethrin which belong to pyrethroid group of insecticide. Test with diagnostic doses of Deltamethrin showed complete susceptibility throughout the three Tehsils of Bajaur agency.

Conclusions

Resistance to insecticides is leading to increase the malaria cases which are an alarming to health problem. Mosquitoes have successfully adapted to most insecticides by becoming physiologically or behaviorally Resistant to them. There is urgent need to carry out novel and complementary strategies against mosquitoes born diseases. It is of great importance to know about the mechanisms governing Resistance. This study of insecticide Resistance/susceptibility of *An. stephensi* in Bajaur agency is helpful to overcome on vector control by using suitable insecticides. Insecticide resistance was mainly observed in vector species against 4% DDT, 5% Malathion, 0.05% Bendiocarb and Deltamethrin 0.05% was found susceptible.

Recommendations

The recommendations of the study are to enable the national health planners and policy makers to make better informed decisions and design more effective resistance management strategies, including switching over from one group of insecticides to new one or rotation of insecticides groups, regular operational research and capacity building program. These recommendations provide guidelines for judicious and rational use of residual insecticides for future vector control.

Followings are some recommendations for best vector control in Bajaur agency

1. To ensure joint collaboration between researcher and control services.
2. To assess the cost effectiveness and implementation issues of control measures in
3. Well defined setting.
4. Need for monitoring to check the susceptibility/Resistance periodically.
5. Relevant field persons/Entomologists should involve in vector control program to
6. Reduce malaria in Bajaur agency.
7. Proper trainings though trained persons for IRS is very necessary because there are
8. More Poisoning cases during IRS [30].

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